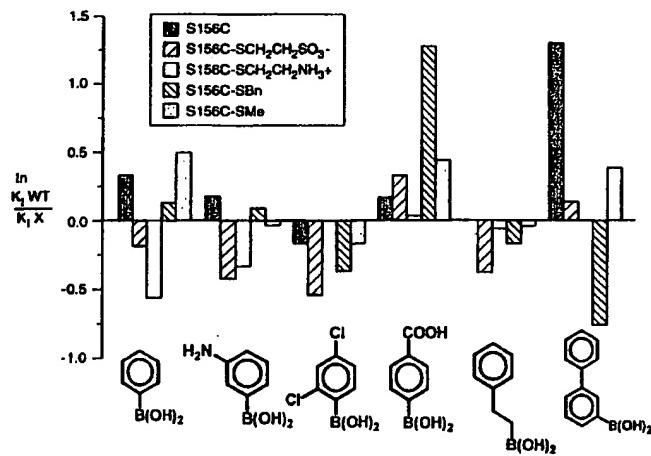




## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                                       |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------|
| (51) International Patent Classification 6 :<br><br>C12N 9/00, 9/54, C11D 3/386                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |  | A3                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | (11) International Publication Number: WO 98/23732<br><br>(43) International Publication Date: 4 June 1998 (04.06.98) |
| <p>(21) International Application Number: PCT/US97/21446</p> <p>(22) International Filing Date: 24 November 1997 (24.11.97)</p> <p>(30) Priority Data:<br/>08/756,664 26 November 1996 (26.11.96) US</p> <p>(71) Applicants: GENENCOR INTERNATIONAL, INC. [US/US]; 4 Cambridge Place, 1870 South Winton Road, Rochester, NY 14618 (US). JONES, J., Bryan [CA/CA]; 1275 Seaford Crescent, Lakefield, Ontario K0L 2H0 (CA).</p> <p>(72) Inventors: BOTT, Richard, R.; 3032 Hillside Drive, Burlingame, CA 94010 (US). GRAYCAR, Thomas, P.; 1166 Manzanita Drive, Pacifica, CA 94044 (US). MITCHINSON, Colin; 381 Myrtle Street, Half Moon Bay, CA 94019 (US). DeSANTIS, Grace; 7 Elm Street #406, Toronto, Ontario M5G 1H4 (CA).</p> <p>(74) Agent: ANDERSON, Kirsten, A.; Genencor International, Inc., 925 Page Mill Road, Palo Alto, CA 94304-1013 (US).</p> |  | <p>(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p><b>Published</b><br/> <i>With international search report.</i><br/> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p> <p>(88) Date of publication of the international search report: 22 October 1998 (22.10.98)</p> |                                                                                                                       |

## (54) Title: CHEMICALLY MODIFIED ENZYME



## (57) Abstract

Modified enzymes are provided in which at least one amino acid, such as asparagine, leucine, methionine or serine, of an enzyme is replaced with a cysteine and the thiol hydrogen is replaced with a substituent group providing a thiol side chain selected from the group consisting of: a)  $-SR^1R^2$ , wherein  $R^1$  is an alkyl and  $R^2$  is a charged or polar moiety; b)  $-SR^3$ , wherein  $R^3$  is a substituted or unsubstituted phenyl; c)  $-SR^4$ , wherein  $R^4$  is substituted or unsubstituted cyclohexyl; d)  $-SR^5$ , wherein  $R^5$  is  $C_{10}-C_{15}$  alkyl; and e)  $-SR^6$  wherein  $R^6$  is a  $C_{1-6}$  alkyl. Also, methods of producing the modified enzymes are provided, as well as detergent and feed additives and a composition for the treatment of a textile. A method for using the modified enzymes in organic synthesis is additionally provided. Further, modified enzymes having improved activity, altered pH profile and/or wash performance are provided.

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# INTERNATIONAL SEARCH REPORT

|                 |                |
|-----------------|----------------|
| Internat'l      | Application No |
| PCT/US 97/21446 |                |

**A. CLASSIFICATION OF SUBJECT MATTER**  
 IPC 6 C12N9/00 C12N9/54 C11D3/386

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                     | Relevant to claim No.       |
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|          | ---                                                                                                                                                                                                                                                                                    | -/-                         |

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

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Date of the actual completion of the international search

5 August 1998

Date of mailing of the international search report

28.08.98

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## INTERNATIONAL SEARCH REPORT

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| Internat        | Application No |
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## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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## INTERNATIONAL SEARCH REPORT

Internati Application No  
PCT/US 97/21446

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                              | Relevant to claim No. |
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## INTERNATIONAL SEARCH REPORT

Int'l application No.  
PCT/US 97/21446

### Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
  
3.  Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

### Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1.  As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
  
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
  
3.  As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
  
4.  No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

#### Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
- No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 2-6, 17-21 34 completely, 1,  
7-12 16 22-27 31-33 partially

Modified enzyme wherein one or more amino acid residues are replaced by cysteine residues, wherin the cysteine residues are modified by replacing the thiol hydrogen with a substituent group of the general formula -SR<sub>1</sub>R<sub>2</sub> in which R<sub>1</sub> is an alkyl and R<sub>2</sub> is a charged or polar moiety.

2. Claims: 35 completely, 1 7-14 16 22-29 31-33 partially

Modified enzyme wherein one or more amino acid residues are replaced by cysteine residues, wherin the cysteine residues are modified by replacing the thiol hydrogen with a substituent group of the general formula -SR<sub>3</sub> in which R<sub>3</sub> is a substituted or unsubstituted phenyl

3. Claims: 36 completely 1 7-14 16 22-29 31-33 partially

Modified enzyme wherein one or more amino acid residues are replaced by cysteine residues, wherin the cysteine residues are modified by replacing the thiol hydrogen with a substituent group of the general formula -SR<sub>4</sub> in which R<sub>4</sub> is substituted or unsubstituted cyclohexyl

4. Claims: 37 38 completely 1 7-12 16 22-27 31-33 partially

Modified enzyme wherein one or more amino acid residues are replaced by cysteine residues, wherin the cysteine residues are modified by replacing the thiol hydrogen with a substituent group of the general formula -SR<sub>5</sub> in which R<sub>5</sub> is C<sub>10-15</sub> alkyl

5. Claims: 15 30 39-42 completely 31-33 partially

Modified enzyme wherein one or more amino acid residues selected from asparagine, leucine or serine are replaced by cysteine residues, wherin the cysteine residues are modified by replacing the thiol hydrogen with a substituent group of the general formula -SR<sub>6</sub> in which R<sub>6</sub> is C<sub>1-6</sub> alkyl.

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

Internati Application No

PCT/US 97/21446

| Patent document cited in search report | Publication date | Patent family member(s) |            | Publication date |
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